

MAJOR REVIEW

Polyamines as key regulatory players in plants under metal stress—A way for an enhanced tolerance

Sofia Spormann | Cristiano Soares  | Jorge Teixeira | Fernanda Fidalgo

GreenUPorto—Sustainable Agrifood
Production Research Centre, Biology
Department, Faculty of Sciences of University
of Porto, Porto, Portugal

Correspondence

Cristiano Soares, Departamento de Biologia,
Faculdade de Ciências, Universidade do Porto,
Rua Campo Alegre s/n, Porto 4169-007,
Portugal.
Email: cfsoares@fc.up.pt

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Abstract

Polyamine (PA) metabolism and functions have started to raise attention from plant scientists in the last years. PAs have been investigated for their involvement in plant cell signalling and protection, proving that alterations in their endogenous levels can affect plant growth, development and survival. The recognised roles of PAs in metal-stressed plants are presented and discussed on a “case-study” basis, for each metal. Bearing in mind that the contamination of soils by heavy metals (HMs) is a growing problem worldwide, it is important to find efficient mechanisms through which agricultural productivity and food quality are safeguarded in a scenario of increased pollution. Making cultivars more tolerant to HM-stress, capable of detoxifying or accumulating them safely, is a goal that will most certainly benefit from researching the functions and applicability of PAs. To date, plant PAs have been recognised for their roles as membrane-, protein- and nucleic acid-stabilisers, as protectors of cellular integrity and photosynthetic machinery, as direct and indirect signalling agents, and as emerging members of the non-enzymatic antioxidant system. Moreover, PAs are not only important in normal plant developmental processes but have also been suggested to induce stress priming, to act as epigenetic regulators of gene expression and to enhance the detoxification and vacuolar compartmentalization of HMs. Although the stress-ameliorating effects of PAs have been widely studied for several abiotic stresses, not much is known regarding their effects on metal induced-stress, except for Cd. This review summarises the available work on the effects of PAs in plants exposed to Cu, Cd, Fe, Mn, Cr, Ni, Hg, Al, Pb and Zn.

KEYWORDS

abiotic stress, antioxidants, heavy metals, oxidative stress, phytoremediation, polyamines

1 | INTRODUCTION

1.1 | Environmental contamination by metals, highlighting the role of polyamines in the stress tolerance response

The current and future trends in population growth come with an increasing demand for food production at a rate that exceeds supply capacity and puts natural resources at risk. Moreover, this

ever-growing demand for food walks parallelly with an unquestionable issue—the effects of climate change and environmental contamination on the biosphere's dynamics. Currently, abiotic stress is recognised as the leading cause of crop productivity losses worldwide. Changes in temperature, precipitation patterns, soil moisture and degradation, including pollution with inorganic and organic contaminants, are already causing serious losses in agricultural yield. Among all abiotic stressors that threaten plants, the contamination of soils/waters by heavy metals (HMs) is of particular significance, because it is a

problem that has been persistently affecting crops and vegetables for several decades now, with consequences additively exacerbated, given the effects of accumulation of these compounds in the soil. Furthermore, it is a relatively challenging problem for producers to deal with, because metal extraction/remediation strategies are still very costly and usage regulations are not yet established.

Alarming levels of HMs are currently present in soils and water used in agricultural practices (Nagajyoti, Lee, & Sreekanth, 2010). As naturally occurring elements, the input of HMs into the environment can be spontaneous, because of pedogenic processes of parent materials' weathering. However, nowadays, the main sources of HM contamination are mostly related to different anthropogenic activities, including those derived from industrial, mining and agricultural practices. As reviewed by Wuana and Okieimen (2011), the use of agrochemicals, including pesticides and fertilisers, and the application of biosolids, manures and wastewaters, along with metal mining processes, leaching of industrial wastes and the presence of airborne contaminants, are the most representative causes of HM contamination. HMs (e.g., Cu, Zn, Ni, Cd, Cr, As, and Hg) comprise metal/metalloid elements presenting a high density ($>5 \text{ g cm}^{-3}$) and an atomic weight above 20 (Rascio & Navari-Izzo, 2011). Despite their important roles on soil composition and nutrient cycles, HMs are non-biodegradable compounds, and their overuse and long-term accumulation in the fields amplify their threats to the growth and productivity of crops and vegetables (Nagajyoti et al., 2010). Although several studies have been set out to find solutions to prevent environmental contamination by HMs and to reduce the agronomical damages from this type of pollution (Baker, Reeves, & Hajar, 1994; Chen, Huang, & Liu, 2009; Clemens, 2001; Giordani, Cecchi, & Zanchi, 2005; Shah & Nongkynrih, 2007; Vinocur & Altman, 2005), the levels of toxic HMs in the environment are still expected to rise in the next decades (Nagajyoti et al., 2010), and the conventional outdoor agricultural facilities are not technologically prepared to overcome their effects, imposing serious risks to local crop yield and food production, given their recognised toxicity for plant organisms. Furthermore, it should be highlighted that the toxicity of HMs depends not only on the chemical nature of the HM (chemical form and speciation), characteristics of the soil environment (pH, humidity and organic matter content) and co-presence of other contaminants (Filipiak-Szok et al., 2015; Pueyo et al., 2004; Wang, Shi, Xu, & Hu, 2007), but also on plant species, sensitivity of organs and tissues, plant age, among others. However, when in excess, all HMs become phytotoxic, hampering plant growth by the interference with different metabolic and physiological processes. One of the most common features of HM toxicity is the appearance of phytotoxic symptoms, like chlorosis and necrosis, accompanied by a great inhibition of plant growth traits (Sharma et al., 2020). Although different HMs may have distinctive effects on plants, differentially affecting the plant organs and metabolic events, it is recognised that their toxicity relies on their interaction with several biomolecules (Seneviratne et al., 2019). Indeed, either by the production of bonds in sulfhydryl (-SH) groups of proteins or by changing the functionality of metals present in the active centre of enzymes and pigments, HMs can majorly disrupt the cellular

homeostasis, inhibiting essential processes such as photosynthesis and aerobic respiration. Besides affecting plant growth and development, metals are widely recognised for disrupting the normal redox balance of cells, favouring the occurrence of oxidative stress (Sharma et al., 2020; Soares, Carvalho, Azevedo, & Fidalgo, 2019). Either directly (e.g., Cu and Fe) or indirectly (e.g., Ni and Cd), high intracellular levels of HMs induce the overaccumulation of reactive oxygen species (ROS), which include both radical (superoxide anion— O_2^- —and hydroxyl radical—OH \cdot) and molecular (hydrogen peroxide— H_2O_2 —and singlet oxygen— $^1\text{O}_2$) forms, ultimately responsible for the oxidation of important organic molecules, such as lipids, proteins and nucleic acids (DNA and RNA) (Soares et al., 2019).

In order to withstand the toxic effects of metal-induced ROS, plants are equipped with a powerful and multifaceted antioxidant (AOX) system, dissected into enzymatic (e.g., superoxide dismutase—SOD; EC 1.15.1.1; catalase—CAT; EC 1.11.1.6; ascorbate peroxidase—APX; EC 1.11.1.11; glutathione reductase—GR; EC 1.6.4.2) and non-enzymatic players (e.g., proline [Pro], ascorbate [AsA], glutathione [GSH], phenols), which synergistically interact to ensure the redox homeostasis by an efficient neutralisation and/or elimination of ROS. However, metal toxicity can also include the impairment of protein activity, usually leading to enzyme inhibition and a lower AOX efficiency (Seneviratne et al., 2019; Sharma et al., 2020). Therefore, the activity of the classic AOX system is not always capable of ensuring the normal redox homeostasis and, therefore, other players must act to prevent losses of cellular functions under HM toxicity. In recent years, an ubiquitous class of compounds has started to raise attention, given their wide spectrum of biological activity. Polyamines (PAs) are water-soluble, low molecular weight polycations, containing two or more amino groups (Kusano, Berberich, Tateda, & Takahashi, 2008). Present in both eukaryotic and prokaryotic cells, PAs possess an aliphatic nitrogenous structure and can be found either in free, covalently conjugated, or non-covalently conjugated forms (reviewed by Chen, Shao, Yin, Younis, & Zheng, 2019 and Rangan, Subramani, Kumar, Singh, & Singh, 2014). Particularly focusing on plants, the main PAs are putrescine (Put; diamine), spermidine (Spd; triamine) and spermine (Spm; tetramine), all being involved in a plethora of phenomena, at both cellular, organ and individual levels. Two other PAs are also commonly found in plant cells: the diamine cadaverine and the Spm structural isomer thermospermine (tSpm; tetramine). Moreover, the distribution of PAs in plant tissues shows differential patterns: while in leaves Put is the most common form, Spd has been shown to be the most prevalent form in other organs (Chen et al., 2019; Takahashi et al., 2018). Subcellularly, PAs can be found in every compartment, including the nucleus (reviewed by Kuznetsov, Radyukina, & Shevyakova, 2006). According to several authors, PAs can have hormone-like features (Todorova, Katerova, Sergiev, & Alexieva, 2014), enabling them to mediate and control several essential processes in plants, spanning from growth (cell division and differentiation) and development (flowering, fruit maturation, organogenesis and embryogenesis) to senescence and stress tolerance (Alcázar et al., 2010; Chen et al., 2019; Gill & Tuteja, 2010; Gupta, Dey, & Gupta, 2013; Kuznetsov et al., 2006; Rangan et al., 2014;

Todorova et al., 2014; Wen & Moriguchi, 2015; Yu, Jia, & Liu, 2019). Within this matter, the accumulation of PAs under stress, as well as their protective role, is of particular interest, given the recognised abilities of these molecules to improve plant tolerance.

Because of the cationic features of PAs, they can easily interact with negatively charged biomolecules, like DNA, RNA and proteins, promoting their stabilisation or destabilisation by the production of electrostatic bonds (Flink & Pettijohn, 1975). Furthermore, recent evidence also strongly suggest that PAs can act as potent AOX, preventing the toxic damages induced by ROS (Soares et al., 2019), not only by modifying the AOX system, given their recognised ability to interact with enzymes, but also by modulating ROS production and scavenge. In fact, as will be discussed later, despite the OH and $^1\text{O}_2$ scavenging ability of PAs, it should be noted that their metabolism also implies the generation of H_2O_2 (Soares et al., 2019).

Although the metabolic pathways of PAs in plants are well described in literature (Alcázar et al., 2010; Chen et al., 2019; Gupta et al., 2013; Tiburcio, Altabella, & Bitrián, 2014; Todorova et al., 2014; Yu et al., 2019), an increasingly growing number of publications evidencing their role in plant abiotic stress tolerance has been noticed in the last years. Ranging from transgenic approaches, where genes involved in PAs metabolism are silenced or overexpressed, to the exogenous application of these polycations, the important role of PAs in the defence of plants against drought (Hassan, Ali, & Alamer, 2018), salt (Baniasadi, Saffari, & Maghsoudi Moud, 2018), temperature (Jing, Guo, Li, & Li, 2020) and metals (Hasanuzzaman et al., 2019; Nasibi, Heidari, Asrar, & Mansoori, 2013; Pathak, 2018) has been recently highlighted. In this review, focus was preferentially given to the role of PAs as ameliorator agents against metal toxicity, especially concentrating on the interplay between PAs, metals and the redox homeostasis. In the subsequent sections, a brief overview of PAs metabolism and biological functions under homeostatic conditions is presented, followed by a description of the main biochemical and molecular

adjustments induced by PAs in HM-exposed plants. Aiming to place readers onto a more practical concept, the last section of this review discusses the suitability of PAs to be used in phytoremediation programs.

2 | PAs—BIOSYNTHETIC AND CATABOLIC PATHWAYS AND BIOLOGICAL FUNCTIONS

2.1 | Synthesis and catabolism of PAs

The diamine Put is the first PA to be formed and is the central product in the PAs biosynthetic pathway, serving as a precursor to Spd and Spm (Chen et al., 2019; Rangan et al., 2014). As represented in the right side of Figure 1, there are three different pathways for Put production in plant cells. The most common route, starting from arginine (Arg), consists of three enzymatic steps catalysed by the sequential action of Arg decarboxylase (ADC), agmatine iminohydrolase (AIH) and N-carbamoylputrescine amidohydrolase, producing agmatine (Agm), N-carbamoyl-Put and Put, respectively, along with CO_2 and NH_3 . In the second route, Arg is converted into ornithine (Orn), through the action of arginase, and Put is formed by the decarboxylation of Orn by the Orn decarboxylase (ODC), releasing CO_2 as well (Chen et al., 2019; Gill & Tuteja, 2010; Hasanuzzaman et al., 2019; Todorova et al., 2014). Curiously, the gene encoding for the ODC enzyme has been lost in many members of the *Brassicaceae* family, including *Arabidopsis thaliana*, which only has one possible route for Put biosynthesis (Chen et al., 2019; Hanfrey, Sommer, Mayer, Burtin, & Michael, 2001). There is also a rare alternative third route in which Arg is converted to citrulline (Cit), which is then decarboxylated by Cit decarboxylase to form Put, but, until now, this pathway has only been described in sesame plants (Chen et al., 2019). The freshly formed Put serves as the precursor for other PAs by the action of the

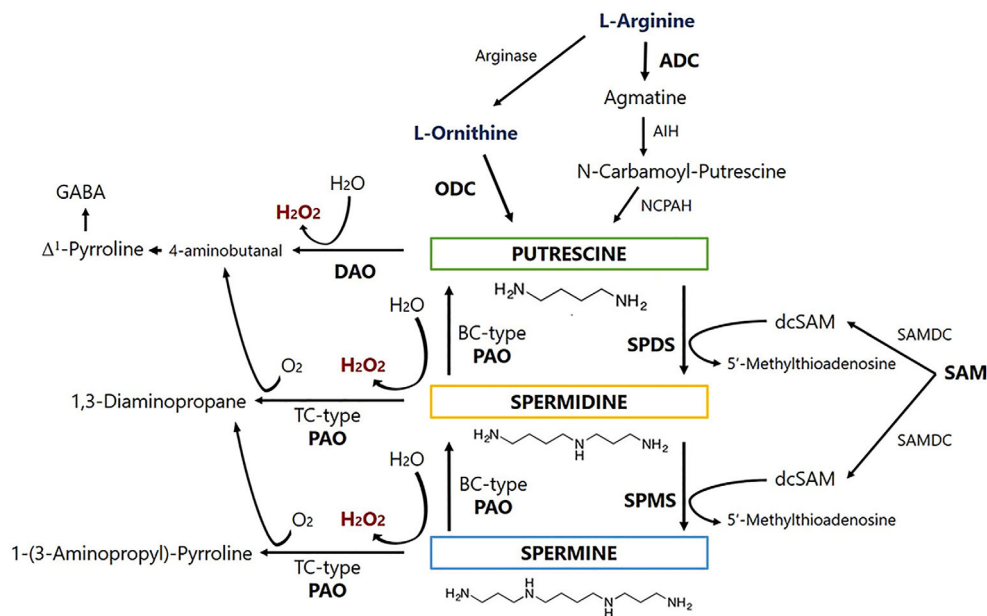


FIGURE 1 Schematic representation of the biosynthesis, back-conversion and terminal catabolism of Put, Spd and Spm in plants. Adapted from Alcázar et al. (2006), Chen et al. (2019), Gill and Tuteja (2010), Rangan et al. (2014), Todorova et al. (2014) and Yu et al. (2019)

enzymes Spd synthase (SPDS) and Spm synthase (SPMS), which form Spd and Spm by the sequential addition of aminopropyl groups to Put and Spd, respectively, being these residues gradually provided by methionine, such as decarboxylated S-adenosylmethionine (dcSAM) (Figure 1) (Alcázar et al., 2006; Chen et al., 2019; Rangan et al., 2014). Further details regarding the biosynthetic pathway of PAs can be found in the outstanding literature reviews that are on the bases of the present work, and whose reading is highly recommended (Chen et al., 2019; Gill & Tuteja, 2010; Gupta et al., 2013; Hasanuzzaman et al., 2019; Rangan et al., 2014; Todorova et al., 2014). Moreover, as mentioned by Wang, Paschalidis, Feng, Song, and Liu (2019), despite the large body of literature on PA biosynthesis, information on their catabolism is still scarce, being most enzymes and genes involved in this process poorly characterised in most model plants.

It is known that the catabolism of PAs depends mainly on the action of two types of amine oxidases (reviewed by Wang et al. (2019) and Yu et al. (2019)). The ones responsible for Put oxidation (as well as for the oxidation of cadaverine) are called diamine oxidases or copper dependent amine oxidases (DAO/CuAO, EC 1.4.3.-), which have a high affinity for diamines, being able to catabolise their oxidation at the primary amino group. CuAOs need to bind to Cu or pyridoxal phosphate as cofactors, and their catabolic activity leads to the formation of 4-aminobutanal (which spontaneously cyclizes to Δ^1 -Pyrroline), H_2O_2 and NH_3 , as represented in Figure 1. Despite their high affinity for diamines, it has also been demonstrated that some CuAOs in *A. thaliana* are also able to oxidise the triamine Spd (Wang et al., 2019). CuAOs can be divided into two groups concerning their subcellular location: some are typically found in the apoplast and carry an N-terminal signal peptide, and the other CuAOs are located to the peroxisomes and carry a C-terminal peroxisomal targeting signal (PTS1) (Wang et al., 2019).

The second type of amine oxidase is called flavin dependent polyamine oxidases (PAO, EC 1.5.3.-). These are responsible for oxidising Spd and Spm, along with their derivatives, at the secondary amino group (Wang et al., 2019; Yu et al., 2019). PAOs link to flavin adenine dinucleotide (FAD) as a cofactor, and are predominantly found in monocot species, while dicots are usually richer in DAOs (Chen et al., 2019; Rangan et al., 2014). PAOs can be divided into two functionally different groups, as is demonstrated in Figure 1. The first group catalyses the oxidation and decomposition of Spd and Spm producing H_2O_2 , 1,3-diaminopropane (DAP), and 4-aminobutanal or N-(3-aminopropyl)-4-aminobutanal, whether it refers to Spd or Spm catabolism, respectively. This class of PAOs are usually referred to as terminal catabolism-type PAOs (TC-type PAOs). The second class of PAOs is involved in a process called PA back-conversion, in which the oxidation of Spm leads to Spd and Spd to Put, in a reverse PA synthesis reaction, which produces 3-aminopropanal and H_2O_2 (Wang et al., 2019). Then again, these PAOs are called back-conversion-type (BC-type PAOs). Following a similar distribution pattern as the two types of CuAOs, TC-type PAOs, involved in the oxidation and decomposition of PAs, are also located in the apoplast, while the BC-type PAOs are usually present intracellularly, in the peroxisomes (Wang et al., 2019). The compartmentalization of PAs could play a significant

role in regulating the rate of their catabolism, because CuAOs and PAOs have a well-defined pattern of distribution, which could be related to specific physiological processes (e.g., lignification or suberization responses in the apoplast) (Rangan et al., 2014), through the production of H_2O_2 , which is a widely recognised signalling molecule with important functions in cell wall maturation processes and stress responses (Yu et al., 2019). Interestingly, despite the high levels of PAOs found in plant tissues, so far, only six genes encoding TC-type PAOs have been identified, while most identified PAOs in monocots and dicots belong to the BC-type. PAOs show different substrate specificities as well as different required conditions of temperature and pH for each substrate (Rangan et al., 2014; Wang et al., 2019).

2.2 | General functions of PAs in the cellular metabolism

PAs are known to be involved in plant growth and development and in the regulation of many basic cellular processes, including DNA replication, transcription, translation, cell proliferation, modulation of enzyme activities, cellular cation-anion balance and membrane stability (Gill & Tuteja, 2010).

PAs are mainly present in their free form, but they can be found covalently conjugated with small molecules such as hydroxycinnamic or phenolics, forming perchloric acid-soluble conjugated PAs (PS-conjugated PAs) (Gill & Tuteja, 2010; Liu, Dong, Zhang, Liu, & Liu, 2004). Insoluble PA conjugates are formed by the action of the transglutaminase enzyme. They are covalently bound to macromolecules, such as proteins, DNA or RNA, forming perchloric acid-insoluble bound PAs (PIS-bound PAs). The interaction with such macromolecules, as well as with the osmolyte Pro and compatible solute γ -aminobutyric acid (GABA), allows PAs to play a role in many important growth and developmental processes in plants (Hasanuzzaman et al., 2019; Podlešáková, Ugena, Spíchal, Doležal, & De Diego, 2019; Todorova et al., 2014).

2.3 | PAs and their role in plant stress responses

The very first insight on the relationship between PAs and stress responses in plants dates back to the 1950s, where Richards and Coleman (1952) observed the occurrence of increased levels of Put in barley plants (*Hordeum vulgare* L.) grown under a potassium (K) deficient medium. Since then, growing evidence has been linking PAs with an enhanced tolerance of plants to different kinds of adverse conditions. Either by the use of mutants or by applying exogenous PAs and/or their inhibitors, recent studies have confirmed that plant PAs are involved in the acquisition of tolerance to multiple abiotic stresses (Gill & Tuteja, 2010), such as high and low temperatures, salinity, hypoxia and soil/atmospheric pollutants, including HMs (Chen et al., 2019; Gupta et al., 2013; Kuznetsov et al., 2006; Paul, Banerjee, & Roychoudhury, 2018; Rangan et al., 2014; Todorova et al., 2014).

In what concerns PAs metabolism, gene expression analysis has shown that ADC-dependent PAs biosynthesis responds much more strongly to abiotic stress than the ODC pathway (Berberich, Sagor, & Kusano, 2015; Do et al., 2013). Concomitantly, and reiterating the key role of PAs in the normal plant metabolism, several studies have been observing differential expression patterns of PAs-related genes, not only those involved in their biosynthesis, but also those targeting proteins linked to PAs catabolism (Gupta et al., 2013). In early work conducted with *Brassica juncea* L. (Indian mustard), Southern blotting techniques revealed that ADC homologues genes (*MADC*) were selectively expressed according to the stress condition (Mo & Pua, 2002). In fact, while *MADC1* was preferentially expressed under chilling, *MADC2* and *MADC3* were also detected under salt and control conditions (Mo & Pua, 2002). In almost cases, evidently, the upregulation of genes involved in PAs metabolism reflects in their abundance and diversity in plant tissues, generally increasing the total content of these polycations in cells (Gupta et al., 2013).

Additionally, it has been stated that PAOs also respond to abiotic stresses (heat, wound, cold, drought, salt and metal toxicity), ROS, phytohormones, as well as PAs levels, implying that these catalytic enzymes could also have various functions in stress tolerance (Hao et al., 2018). The expression and activity patterns of PAOs and CuAOs have been the research focus of several studies in different plant species (reviews by Gupta et al., 2013; Gupta, Sengupta, Chakraborty, & Gupta, 2016). According to Moschou et al. (2008), a higher catabolic index of PAs results in a higher production of H_2O_2 , being its levels determinant for its function. While at some extent, PAO-induced H_2O_2 may function as an important signalling molecule, leading to the activation of stress responses, upon several conditions, the excess of this ROS can result in programmed cell death (PCD). Therefore, plants must tightly coordinate PAs anabolic and catabolic pathways in order to ensure the cellular homeostasis under stress. Actually, sensitive species under abiotic stress witness a marked increase in the levels of PAs, which are secreted in the apoplast and boost the activity of PAOs and CuAOs therein, leading to a higher production of H_2O_2 , threatening the redox homeostasis of cells. In fact, the PCD response can be activated under severe stress because of excessive PAs catabolism, if the levels of ROS produced by DAOs and PAOs exceed a specific threshold and lead to the downregulation of pro-survival genes (Wang et al., 2019). If under mild stress, however, plants might overcome the induced ROS burst with a little help from Pro, GSH and other AOXs, which are also induced by stress and/or by PAs accumulation and catabolism (Yu et al., 2019).

Although in some cases higher levels of PAs in the cells are correlated to higher stress tolerance (Hasanuzzaman et al., 2019; Tajti, Janda, Majláth, Szalai, & Pál, 2018), this relationship should not be so simplistically generalised. The three main plant PAs appear to have different functions under abiotic stress. On sensitive plant species, Put content usually increases quickly as a response to changes in the environment, which reflects in a decreased (Spm + Spd)/Put ratio, being these changes usually accompanied by the generation of ROS, which, altogether, is considered as a stress signal (Groppa & Benavides, 2008; Paul et al., 2018; Zhao & Yang, 2008). In contrast,

stress-tolerant species and cultivars are usually able to maintain higher levels of Spd and Spm under stress, while Put levels remain relatively low, which could imply their higher resilience (Sánchez-Rodríguez, Romero, & Ruiz, 2016). Excessive Put accumulation in cells under stress can cause serious negative effects, such as the depolarization of membranes, leading to potassium leakage, tissue necrosis and protein loss, especially in leaf tissues. On the other hand, Spd and Spm have anti-senescence effects under stress, being crucial for preserving the integrity of thylakoid membranes (Zhao, Shi, & Yuan, 2008). A more precise relationship between PAs levels and stress tolerance has been suggested, in which increases in the (Spm + Spd)/Put ratio are behind the resistance to abiotic stresses such as drought and HM-stress (Sánchez-Rodríguez et al., 2016; Wang et al., 2007; Zhao & Yang, 2008), although this logic cannot be applied to every species or stress factor. For example, according to Do et al. (2013), in rice plants, under control conditions, Put is the predominant PA, followed by free Spd and Spm, but under drought stress, Put levels decrease and Spm becomes the most prominent PA. In fact, the induction of PAs biosynthesis differs not only between different plant species but also between different plant organs and growth conditions. It has been observed that the expression of several genes involved in the biosynthesis of PAs is strongly induced by one or more abiotic stresses. In fact, stress- and abscisic acid (ABA)-responsive elements have been found in the promoter sequences of ADC, AIH, CPA, SAMDC, SPDS, SPMS and ACL genes from *A. thaliana* (Alcázar et al., 2006).

The fact that PAs metabolism overlaps other metabolic chains in plant cells increases the complexity of their involvement as signalling molecules. PAs are known to interact with different chemical compounds, including nitric oxide (NO), amino acids (e.g., GABA and Pro) and even phytohormones, such as ethylene and ABA. Actually, genetic analysis of PAs-related genes revealed the presence of ABA-responsive elements, suggesting that this phytohormone may play a role in PAs levels, especially in stress conditions (Gupta et al., 2013; Minocha, Majumdar, & Minocha, 2014; Pál, Szalai, & Janda, 2015). ABA-deficient and -insensitive mutants of *A. thaliana* showed a downregulation of genes coding for ADC2, SPDS1 and SPMS, while in wildtype plants their expression was boosted by drought (Alcázar et al., 2006). Likewise, in fava bean, data showed that, under chilling stress, ABA levels rose in parallel with an increase of Put content (An et al., 2008). Thus, it appears that there might be a positive feedback loop between PAs and ABA metabolism (Pál et al., 2017). The existence of a crosstalk between PAs and GABA under homeostatic and stress circumstances is also widely accepted. As reviewed by Podlešáková et al. (2019), the endogenous levels of GABA under stress are most likely related to the catabolism of PAs, because of the higher activity of PAOs. On the other hand, as reported by Shi and Chan (2014), the exogenous application of GABA to plants alters the expression profile of PAs biosynthetic genes under stress. Another example elucidating the signalling role of PAs is their involvement on the epigenetic regulation of gene expression (Paul et al., 2018). For instance, PAs have been shown to modulate chromatin structure. Their attachment to chromatin can disrupt the access of polymerases

to certain genomic regions and lead to changes in gene expression. In addition, PAs are able to interact with chromatin-modifying and remodelling agents and are also able to promote or inhibit enzymatic activity, leading to histone post-translational modifications like acetylation, deacetylation, methylation, demethylation, and so forth (reviewed by Paul et al. (2018) and Tiburcio et al. (2014)). The patterns of DNA methylation, required for the tight regulation of gene expression, can also be affected by the action of PAs. For example, it has been observed that when Spd and Spm levels increase, the levels of the methyl donor SAM also increase, leading to DNA methylation and possibly gene silencing (Tiburcio et al., 2014). PAs are also reported to upregulate the activity of protein kinases in plants under stress (Pál et al., 2015). It is believed that the interaction of PAs with chromatin and DNA inculcates “stress memory” in plants (Paul et al., 2018), because many of these post-translation modifications can be permanent and maintained for the generations to come. In addition, PAs can also bind directly to DNA or RNA molecules, resulting in either increased stability or induction of structural changes that later reflect on disturbances in transcription and translation, respectively (Lightfoot & Hall, 2014). As it has been observed that there are specific responses associated with levels of certain PAs, the existence of specific mechanisms has been suggested, covering the selective recognition of target genes or genomic regions by each type of PAs. In this sense, the presence of PA-responsive elements in the promoter sequences of stress-responsive genes induced by them is being investigated (Pál et al., 2015). A new concept is being introduced in this research field. The term “PA modulon” was firstly proposed by Yoshida et al. (2004), upon isolating a set of bacterial genes whose translation was enhanced by PAs, being most of these encoding for

transcription factors (Igarashi & Kashiwagi, 2011). The concept of PA modulon was further explored in the reviews by Pegg and Casero (2011), Igarashi and Kashiwagi (2011), Lightfoot and Hall (2014) and Tiburcio et al. (2014), according to whom it is now also being applied and investigated to yeast and mammalian cells. The existence of such modulon in plants requires further investigation; however, the most recent findings seem to suggest that PAs are responsible for regulating the expression and synthesis of genes and proteins, namely those related to hormone metabolism and signalling and to stress responses (Tiburcio et al., 2014).

Despite the important roles PAs have as intermediate signalling agents in a wide range of metabolic chains, including those related to NO, ABA, GABA and ethylene, the overaccumulation of these aliphatic molecules in plant cells under stress can also directly control the maintenance of the redox homeostasis, by preventing the occurrence of oxidative stress, as pointed out in Figure 2 (Gill & Tuteja, 2010; Groppa & Benavides, 2008; Soares et al., 2019; Todorova et al., 2014). Being part of the non-enzymatic component of the plant AOX system, their ameliorating effects on ROS overaccumulation is much likely related to their chemical features, combining their acid-neutralising anionic/cationic-binding properties (Gupta et al., 2013). However, as reviewed by Minocha et al. (2014), the exact points of interaction between PAs and ROS are far from completely understood and remain as one of the most curious and complex biochemical phenomena occurring in plant cells. This fog of knowledge that hangs over the interaction of PAs with ROS arises mainly from the fact that, if on the one hand, PAs can directly eliminate ROS, such as $^1\text{O}_2$ and OH $^\cdot$, on the other hand, their catabolism results in the production of oxidative species, namely H_2O_2 (Minocha et al., 2014).

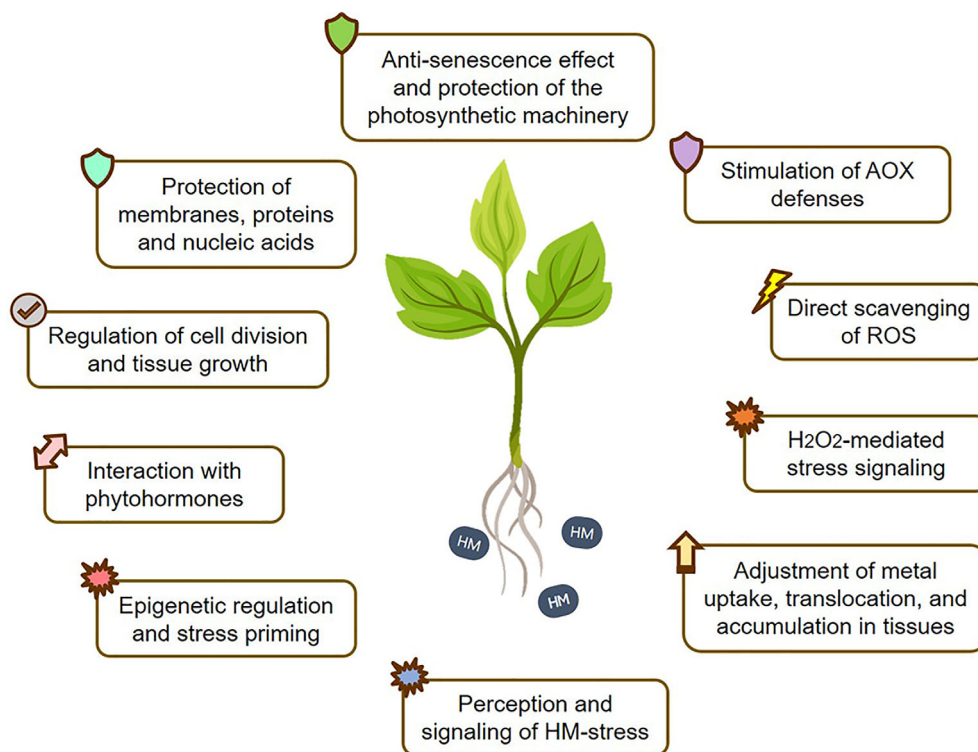


FIGURE 2 The diverse roles of PAs in plants under HM stress

Still, the great majority of studies dealing with PAs and stress-exposed plants unequivocally elucidates the powerful action of these compounds as AOX, which can simultaneously act as radical scavengers, membrane stabilisers and inhibitors of lipid peroxidation (Alcázar et al., 2006; Gill & Tuteja, 2010; Groppa & Benavides, 2008; Gupta et al., 2013; Sánchez-Rodríguez et al., 2016; Yu et al., 2019).

Indeed, the protective effect of PAs against stress-induced oxidative burst is well documented for a wide range of adverse growth conditions, including drought, salinity, ultraviolet radiation, extreme temperatures, mineral disbalances and HMs excess (Berberich et al., 2015; Groppa & Benavides, 2008; Groppa, Tomaro, & Benavides, 2001, 2007; Gupta et al., 2013; Jing et al., 2020; Liu et al., 2019). For instance, and concentrating on brand new studies, Baniasadi et al. (2018) and Hassan et al. (2018) reported that the exogenous application of Spd and Spm resulted in a higher tolerance of *Rosa damascena* Miller and *Callendula officinalis* L. to drought (50% field capacity) and salt (5 and 9 dS/m) conditions, respectively. In both studies, performed with different plant species and stress factors, the foliar treatment of plants with PAs helped to re-establish the normal redox balance of the cells, lowering the content of lipid peroxidation and the production of ROS and increasing the efficiency of the plant AOX system. Furthermore, upon exposure to heat stress, tomato (*Solanum lycopersicum* L.) seedlings stimulated the production of all the three PAs (Put, Spd and Spm), with a parallel increase in the transcript levels of the main PAs biosynthetic enzymes—ADC1, ADC2 and ODC1 (Jahan et al., 2019). Also exploring the physiological impacts of elevated temperatures, Jing et al. (2020) reported that the effects of Spm and Spd foliar application in two varieties of wheat (*Triticum aestivum* L.) were time-dependent, showing a dynamic response. Even so, the applied approach helped to diminish grain damages in the two wheat varieties under heat stress, with a substantial enhancement of the AOX potential (evidenced by a higher activity of SOD, POD and CAT) and a reduction of malondialdehyde (MDA), one of the main subproducts of lipid peroxidation. In fact, the possible role of PAs in limiting the peroxidation of lipids might arise from their ability to create a complex between phospholipids and iron, preventing its autoxidation (Hasanuzzaman et al., 2019) and, thus, the consequent formation of OH[•] by the Haber-Weiss cycle (Soares et al., 2019).

Globally, it is widely accepted that PAs can be efficient for plants to cope with environmental stresses, especially in the maintenance of the redox balance of the cells, as illustrated in Figure 2. Biochemical, molecular and bioaccumulation data suggest that plant cells can buffer PA levels at both control and stress conditions, leading to both stress and tolerance responses (Alcázar et al., 2010; Gill & Tuteja, 2010; Todorova et al., 2014). Regarding metal-exposed plants, the abovementioned patterns are also often observed, with a general increase of PAs accumulation and an overstimulation of the main AOX defences, contributing for a better tolerance index. It has been suggested that the coordinated levels of PAs, Ca²⁺, ABA and H₂O₂ present a key adaptation response of plants under several environmental stresses (Wang et al., 2019). However, the combined action of PAs and brassinosteroids can also modulate the levels of other AOXs like GSH, AsA, Pro and glycine-betaine and the activities of enzymes

like GR, SOD, CAT, APX, peroxidase (POX, EC 1.11.1.1) and guaiacol peroxidase (GPOX, EC 1.11.1.7), having possible implication on HM stress tolerance (Rangan et al., 2014). Moreover, the conversion of free PAs to conjugated PAs has been shown to improve abiotic stress tolerance in plants (Mauricio et al., 1999), and in a study with *Hydrocharis dubia*, the levels of conjugated PAs were found to be more steady than those of their free forms, in response to Cd and Ni stress (Yang, Shi, Li, & Wu, 2013; Zhao et al., 2008).

Besides the aforementioned protective roles of PAs, such as membrane-, protein- and nucleic acid-stabilising ability, ROS-scavenging power and signalling function for inducing the production of other AOX molecules, novel attributes have been suggested for PAs, specifically in what regards to HM stress (see Figure 2). PAs work as signalling molecules in plants under metal-stress, so they can regulate ion homeostasis and transportation by interacting with ion channels, inhibiting metal accumulation in tissues and promoting the formation of metal conjugates with phytochelatins (PCs) (Hasanuzzaman et al., 2019). HM conjugation with PCs is one of the most efficient metal detoxification mechanisms in plants (Hasanuzzaman et al., 2019). The induction of PCs synthesis under metal stress occurs because of enhanced activity of the enzyme PC synthase, which uses GSH molecules as precursors to form PCs. PAs play a role in increasing the levels of GSH under metal stress and, consequently, stimulating the production of PCs and PC-conjugates, which are then redirected to the vacuoles (Hasanuzzaman et al., 2019). Kuznetsov's team have also reported that the PAs per se could form metal chelates and that the upregulation of PAs biosynthesis and accumulation in shoot tissues, along with the existence of PA transporters in the vacuolar membrane, could elucidate a role for PAs in the detoxification process of HMs, Ni in particular, with interesting potential for phytoremediation purposes (Kuznetsov et al., 2006; Pietrini et al., 2015; Shevyakova, Cheremisina, & Kuznetsov, 2011; Shevyakova, Il'ina, & Kuznetsov, 2008; Shevyakova, Il'ina, Stetsenko, & Kuznetsov, 2011). This idea is further discussed in Section 5. In the next sections of this review, PAs involvement in HM tolerance will be explored by focusing on different case studies for the most significant HMs present in the environment.

3 | CASE STUDIES ON THE CONTRIBUTION OF PAs IN PLANTS UNDER HM-STRESS

3.1 | Copper (Cu)

Like many other metals, high Cu concentrations cause serious consequences to plants, leading to alterations in morphology and physiology, at both cellular and molecular level (Adrees et al., 2015). Cu is a HM that is usually trapped in the roots of plants instead of being translocated to the upper parts. As a result, the toxicity effects induced by exposure to high levels of Cu are usually more visible in the root system, with a strong growth inhibition and induction of oxidative stress, although chlorosis and necrosis have been observed in

the leaves of plants under Cu toxicity. In response to Cu-stress, several plants show increased root accumulation of Put, along with the often-increased activity of ADC enzyme involved in the biosynthesis of this PA, as reviewed by Paul et al. (2018). The deleterious effects of Cu are thought to be diminished by the potential of PAs to modulate the AOX system (Paul et al., 2018). Choudhary et al. (2009) have shown that Cu toxicity could be ameliorated with the exogenous application of 24-epibrassinolide, partially because of its interaction with the biosynthetic pathways of PAs, IAA and ABA. In line with this, the pre-treatment of wheat plants with 1 mM Spd helped to revert Cu toxicity, by lowering Cu accumulation and oxidative damage and enhancing the AOX efficiency (Agami, 2016). The same pattern has been also observed for other Cu-exposed plants previously treated with different PAs (Table 1), especially Spd and Spm. When studying the phytotoxicity of Cu in *Raphanus sativus*, Choudhary et al. (2012) showed that the application of 0.1 mM Spd could efficiently alleviate the deleterious effects of the HM. Besides limiting Cu uptake, the exogenous applied PAs reduced the extent of oxidative stress, lowering the accumulation of both H_2O_2 and O_2^- .

3.2 | Cadmium (Cd)

Several studies have set out to unravel the involvement of PAs on Cd-induced toxicity to plants. Although Cd is a non-active redox metal, exposure to high levels of this HM causes root and shoot growth inhibition, oxidative stress, reduction in photosynthesis and nutrient uptake, decrease in carbon assimilation and disturbed water balance (Qin et al., 2020). It has been observed, in different plant species, that exposure to high levels of Cd leads to a decreased (Spm + Spd)/Put ratio because of induced accumulation of Put and increased enzymatic activity and/or gene expression of ADC and ODC, DAO and PAO (Paul et al., 2018; Yang et al., 2013). Changes in PIS-bound or PS-conjugated PA levels under Cd can be different from those of free PAs. For example, Yang et al. (2013) suggested that the conversion of free Put (the most toxic form of PA) to PS-conjugated or PIS-bound PAs, or even to free Spd and Spm, could alleviate Cd stress. In the leaves of *H. dubia*, Cd treatment led to an increase in the levels of PIS-bound Put, but not as massively as the accumulation of free Put, while upon applying exogenous Spd, the levels of PS-conjugated and PIS-bound forms of Spd and Spm were greatly enhanced (Yang et al., 2013). Moreover, Cd-stressed plants with increased endogenous levels of PAs have also exhibited a higher AOX potential (Roychoudhury, Basu, & Sengupta, 2012), which could be partly triggered by the signalling role of PAs and consequent H_2O_2 burst (Gupta et al., 2013; Pooja, Devi, & Munjal, 2019; Wang et al., 2019). In Cd-exposed plants, increased levels of Spd and Spm were described as a plant-induced defensive strategy to avoid metal stress (Hasanuzzaman et al., 2019; Howladar, Al-Robai, Al-Zahrani, Howladar, & Aldhebiani, 2018).

In different approaches, it was observed that the exogenous application of PAs to plants under Cd-stress leads to higher resilience, improvement of photosynthetic traits and enhanced ROS scavenging

potential, by promoting the production and activity of AOXs. Moreover, Tajti et al. (2018) reported that a pre-treatment with PAs could confer Cd tolerance to wheat plants, possibly through promoting metal chelation and enhancing defensive mechanisms. As illustrated in Table 1, Cd is the HM for which more works have been conducted so far on the crosstalk between PAs and its effects on plant growth and physiology. Based on the results of Rady and Hemida (2015), seed priming with 2 mM Spd or Spm resulted in a higher tolerance of *T. aestivum* to 1 μ M Cd. The protective effect was ascribed to PA-mediated regulation of the AOX metabolism, because of an overproduction of AOX metabolites and a higher activity of SOD and glutathione-S-transferase (GST) and decrease of the oxidative stress markers (H_2O_2 and MDA content). On the contrary, Pál et al. (2017) found that the exogenous application of Put in rice plants exposed to Cd did not contribute to a better plant performance and even accentuated Cd effects on plant growth (Pál et al., 2017). Groppa, Rosales, Iannone, and Benavides (2008) have also reported that the single exposure to Put or Spm caused an even stronger root growth inhibition to wheat seedlings than Cd-stress itself. Nahar et al. (2016), on the other hand, reported that the exogenous application of Put could improve mung bean plants tolerance to Cd, and they noticed that this protective effect was even better when plants were also treated with sodium nitroprusside (SNP), a NO donor. In fact, it has been reported that NO enhances the AOX defences of Cd-stressed ryegrass plants, similarly to PAs, mitigating the oxidative stress caused by Cd, and even improved the uptake of essential nutrients, and decreased the root-to-shoot translocation of Cd, preventing it from causing further damage to the leaves (Chen et al., 2019). In combination with PAs, it is possible that the protective properties of both molecules might be acting together to help plants overcome stress. Nahar et al. (2016) suggested that a cross-protection phenomenon between PAs and NO is responsible for a higher production of PCs under metal stress and a marked improvement of both AOX metabolites and enzymes, resulting in an overall growth improvement and better metal homeostasis. In fact, the existence of such NO-Pas crosstalk has long been acknowledged given the possible linkage between PAs and NO synthesis, although the mechanism through which PAs induce NO formation is still not fully understood (Tun et al., 2006; Yamasaki & Cohen, 2006; Groppa et al., 2008). The exogenous application of PAs induces the formation of NO (Tun et al., 2006; Groppa et al., 2008), and curiously, exposing plants to NO or Cd also enhances the internal production of PAs (Groppa et al., 2008). These authors have suggested that higher levels of NO mediate the toxicity exerted by Cd and by PAs in wheat plants, leading to a higher AOX potential, but also to higher levels of Cd in the roots, and consequently, to higher root growth inhibitions (Groppa et al., 2008). Similarly, Cd induced the production of NO in roots and leaves of *A. thaliana* seedlings and it was observed that NO contributed to the Cd-triggered inhibition of root growth and Cd-induced inhibition of root Ca accumulation (Besson-Bard et al., 2009). While some authors see NO and PAs as a protective molecules, some studies indicate that NO increases the sensitivity of plants to Cd, and that this effect is further enhanced with PA treatment because of their effect on inducing NO production.

TABLE 1 Case studies on the contribution of PAs in plants under HM-stress. Green and red arrows represent positive and negative effects, respectively

Metal	Concentration	Plant species	PA treatment	Exposure time	Biomarkers	PA levels	Metal accumulation	Oxidative stress	AOX system	Citation
Cu	0.05 mM	<i>Nymphoides peltatum</i>	Spd and Spm (0.1 mM)	4 d	Proline, LP, O ₂ ⁻ , H ₂ O ₂	> (Spd + Spm)/Put	↓	↓	↑	Wang et al., 2007
Cu	0.05–0.2 mM	<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	Spd (0.1 mM)	5 d	LP, O ₂ ⁻ , H ₂ O ₂	< Put; > Spd; = Spm	↓	↓	-	Xu et al., 2011
Cu	0.2 mM	<i>Raphanus sativus</i>	Spd (1 mM)	7 d	O ₂ ⁻ , H ₂ O ₂ , LP, ion leakage, proline, GSH, AsA, phenols, SOD, CAT, APX, GPOX, MDHAR, DHAR, GR	= Put; > Spd; < Spm	↓	↓	↑ (non-enzymatic) Variable (enzymatic)	Choudhary, Kanwar, Bhardwaj, Yu, & Tran, 2012
Cu	100 μM	<i>Chlorella vulgaris</i>	Spd (100 μM)	24 hr	LP, GSH, AsA, SOD, CAT, APX	-	=	↓	↑	Piotrowska-Niczyporuk, Bajguz, Zambrzycka, & Godlewska-Żyłkiewicz, 2012
Cu	0.5 mM	<i>Helianthus annuus</i> L.	Spd and Spm (1 mM)	14 hr	LP, SOD, APX, GR	> Spm and Spd	-	↓	↑↓	Groppa et al., 2001
Cu	0.5 mM	<i>Triticum aestivum</i> L.	Spd and Spm (1 mM)	14 hr	LP, H ₂ O ₂ , SOD, APX, GR, GPOX, GSH	> Put, Spm and Spd	-	↓	↑↓	Groppa et al., 2007
Cu	250, 500 μM	<i>Triticum aestivum</i> L.	Spd (1 mM)	60 d	Electrolyte leakage, Proline, APX, SOD, GPOX	-	↓	↓	↑	Agami, 2016
Cd	1.0 mM	<i>Triticum aestivum</i> L.	Spd and Spm (2.0 mM)	45 d	LP, H ₂ O ₂ , AsA, GSH, Proline, CAT, APX	> Spd and Spm	↓	↓	↑↓	Rady & Hemida, 2015
Cd	150 and 300 μM	<i>Malus hupehensis</i> Rehd.	Spd and Spm (250 μM)	3 d	MDA, O ₂ ⁻ , SOD, GPX	> (Spd + Spm)/Put	-	↓	↑	Zhao & Yang, 2008
Cd	1.5 mM	<i>Vigna radiata</i>	Put (0.2 mM)	2 d	H ₂ O ₂ , O ₂ ⁻ , LP, Proline, AsA, GSH, PC, SOD, CAT, MDHAR, DHAR, GR, GST	> (Spd + Spm)/Put	↓	↓	↑	Nahar et al., 2016
Cd	50 μM	<i>Oryza sativa</i> L.	Put (0.5 mM)	7 and 14 d	H ₂ O ₂ , GSH, PC, GPOX, GR	> Put, Spd and Spm	=	↑	↑↓	Pál et al., 2017
Cd	0.1, 0.2 mM	<i>Typha latifolia</i> L.	Spd (0.25 and 0.50 mM)	3 d	O ₂ ⁻ , H ₂ O ₂ , MDA, GSH, AsA, SOD, CAT, APX, GR, GPOX	-	-	↓	↑↓	Tang et al., 2005

(Continues)

TABLE 1 (Continued)

Metal	Concentration	Plant species	PA treatment	Exposure time	Biomarkers	PA levels	Metal accumulation	Oxidative stress	AOX system	Citation
Cd	0.04 mM	<i>Hydrocharis dubia</i> (Bl.) Backer	Spd (0.1 mM)	6 d	LP, O ₂ ⁻ , H ₂ O ₂	> (Spd + Spm)/Put	-	↓	-	Yang et al., 2013
Cd	5.0 mM	<i>Oryza sativa</i> L.	Spd and Spm (5.0 mM)	6 hr (PA) + 18 hr (Cd)	H ₂ O ₂ , LP, SOD, APX, AsA, GSH, GR, GPOX	-	↓	↓	↓	Hsu & Kao, 2007
Cd	2.0 mM	<i>Triticum aestivum</i> L.	Spm (0.25 mM), Spd (0.50 mM) and Put (1.0 mM)	75 d	SOD, CAT, POD, GR, DPPH,	-	↓	-	↑	Taie, Seif El-Yazal, Ahmed, & Rady, 2019
Cd	0.05, 0.10 mM	<i>Salix matsudana</i>	Spd (0.25 mM)	23 d	O ₂ ⁻ , H ₂ O ₂ , GSH, SOD, CAT, GPX, GR	> Spd; < Put	↓	↓	↑	Tang et al., 2019
Cd	1.0 mM	<i>Triticum aestivum</i> L. and <i>Helianthus annuus</i> L.	Put, Spd and Spm (0.1 mM)	15 d	Membrane fluidity	-	-	↓	-	Benavides, Groppa, Recalde, & Verstraeten., 2018
Cd	1.0 mM	<i>Triticum aestivum</i> L.	Spd and Spm (2.0 mM)	45 d	Electrolyte leakage, H ₂ O ₂ , LP, Proline, GSH, AsA, CAT, APX, POX	> Spd and Spm	↓	↓	↑↑	Rady & Hemida, 2015
Cd	100 μM	<i>Chorella vulgaris</i>	Spd (100 μM)	24 hr	LP, GSH, AsA, SOD, CAT, APX	-	=	↓	↑	Piotrowska-Niczyporuk et al., 2012
Cd	0.5 mM	<i>Helianthus annuus</i> L.	Spd and Spm (1 mM)	14 hr	LP, SOD, APX, GR	> Spm and Spd	-	↓	↑↓	Groppa et al., 2001
Cd	0.5 mM	<i>Triticum aestivum</i> L.	Spd and Spm (1 mM)	14 hr	LP, H ₂ O ₂ , SOD, APX, GR, GPOX, GSH	> Put, Spm and Spd	-	↓	↑↓	Groppa et al., 2007
Cd	50 μM	<i>Triticum aestivum</i> L.	Put and Spd (0.5 mM)	7 d	Proline, APX, CAT, GPOX, GR, GST	= Spd and Put, < Spm	↑	-	↑↓	Tajti et al., 2018
Cd	45 μM	<i>Boehmeria nivea</i> L.	Spd (0.1 mM)	7 d	H ₂ O ₂ , LP, Vitamin E, SOD, POD	-	↓	↓	=	Gong et al., 2016
Ni	125–1,000 μM	<i>Brassica napus</i> L.	Put (1 mM)	5 d	LP	< Put	↑	-	-	Shevyakova, Cheremisina, & Kuznetsov, 2011
Ni	200 mg/kg	<i>Cajanus cajan</i>	Put, Spd and Spm (0.5 mM)	80 d	Membrane stability	-	↓	↓	-	Garg & Saroy, 2020
Zn	200 mg/kg	<i>Vigna radiata</i> L.	Spd (1 mM)	30 d	-	-	-	-	↑	Mir et al., 2016

TABLE 1 (Continued)

Metal	Concentration	Plant species	PA treatment	Exposure time	Biomarkers	PA levels	Metal accumulation	Oxidative stress	AOX system	Citation
Al	0.5 mM	<i>Vigna radiata</i> L.	Spd (0.3 mM)	48 and 72 hr	Proline, SOD, CAT, GPOX; O ₂ ⁻ , H ₂ O ₂ , LP, Proline, AsA, GSH, SOD, CAT, GST, GPX	> (Spd + Spm)/Put	-	↓	↑	Nahar, Hasanuzzaman, Suzuki, & Fujita, 2017
Al	480 μM	<i>Salvinia natans</i> L.	Put (1 mM)	7 d	O ₂ ⁻ , H ₂ O ₂ , LP, protein oxidation, GSH, GPOX, APX, CAT, GR, SOD	-	↓	↓	↑↓	Mandal, Ghosh, Dey, & Adak, 2013
Al	30 μM	<i>Triticum aestivum</i> L.	Put (2 mM)	12 hr	Cell death, H ₂ O ₂ , LP, LOX, SOD, CAT, GPOX, GST, GR	> Spd and = Put	-	↓	↓	Yu et al., 2018
Al	50 μM	<i>Phaseolus vulgaris</i> L.	Put (1 mM)	12 and 24 hr	O ₂ ⁻ , H ₂ O ₂ , LP	> Put	↓	↓	-	Wang, Huang, W. Liang, B. Liang, & Bi, 2013
Hg	10–40 μM	<i>Eichornia crassipes</i> Mart.	Spd (0.1 mM)	6 d	Proline,	> (Spd + Spm)/Put	-	-	↑	Ding, Shi, Xu, Yang, & Xu, 2010
Cr	1.2 mM	<i>Raphanus sativus</i> L.	Spd (1 mM)	7 d	O ₂ ⁻ , H ₂ O ₂ , LP, ion leakage, GSH, Proline, AsA, GB, Phenols, SOD, CAT, GR, GPOX	> Put; < Spd	-	↓	↑↓	Choudhary et al., 2012
Mn	30 and 150 mg/kg	<i>Brassica juncea</i> L.	Spd and Put (1 mM)	10 d	O ₂ ⁻ , H ₂ O ₂ , ion leakage, Proline, CAT, POX, SOD	-	↓	↓	↑	Hussain, Nazir, & Fariduddin, 2019b
Pb	2.0 mM	<i>Triticum aestivum</i> L.	Put (1 mM), Spd (0.5 mM), Spm (0.25 mM)	75 d	Ion leakage, membrane stability, Proline	-	↓	↓	↑	Rady, Seif El-Yazal, Taie, & Ahmed, 2016
Pb	2.0 mM	<i>Triticum aestivum</i> L.	Put (1 mM), Spd (0.5 mM), Spm (0.25 mM)	75 d	SOD, CAT, POD, GR, DPPH,	-	-	-	↑	Taie et al., 2019
Pb	100 μM	<i>Chorella vulgaris</i>	Spd (100 μM)	24 hr	LP, GSH, AsA, SOD, CAT, APX	-	↓	↓	↑	Piotrowska-Niczyporuk et al., 2012

3.3 | Chromium (Cr)

Being a non-essential HM, Cr-induced stress leads to severe physiological and developmental disturbances, including redox imbalances, ultrastructural changes in the chloroplasts and other subcellular damages, and disturbances to the photosynthetic machinery (Hayat et al., 2012). Cr toxicity has also been related to adjustments in PAs metabolism (Paul et al., 2018). Similar to Cd and other HMs, Cr exposure also causes a higher accumulation of free Put, rather than Spd or Spm, along with higher enzymatic AOX activity and Pro levels (Scoccianti, Bucchini, Iacobucci, Ruiz, & Biondi, 2016). In some species a relationship between increased levels of Put or Spd (and SPDS activity) and protection against Cr-stress could be found (Paul et al., 2018). Cr-toxicity has been reported to enhance the levels of free, soluble-conjugated and insoluble-bound PAs in leaves of Kinnow mandarin grafted plants, because of tight regulation of the PAs anabolic and catabolic enzymes (Hasanuzzaman et al., 2019; Shahid et al., 2018). Furthermore, the exogenous application of Spd, in combination with 24-epibrassinolide, has been shown to help plants overcome Cr-induced stress (Choudhary et al., 2012), through improving the redox homeostasis and modulating the activity of PAs biosynthetic enzymes. The combined action of 24-epibrassinolide and Spd led to reset the levels of photosynthetic pigments and significantly increase quantum yield in Cr-stressed seedlings (Choudhary et al., 2012).

3.4 | Nickel (Ni)

Excess of Ni can harshly affect photosynthesis, mineral nutrition, photo-assimilate transport and enzymatic activity. Hence, Ni excess often leads to growth inhibition and induces several toxicity symptoms like chlorosis, necrosis and leaf wilting. Furthermore, Ni-mediated stress is linked to the establishment of oxidative conditions, even though Ni is not a redox-active metal. In the case of Ni-stress, there are only few records discussing the roles of PAs as mediators of plant tolerance (Table 1). Even yet, as mentioned before, a positive action of PAs has been suggested for a long time now by Kuznetsov's team (Kuznetsov et al., 2006; Pietrini et al., 2015; Shevyakova et al., 2008; Shevyakova, Cheremisina, & Kuznetsov, 2011; Shevyakova, Il'ina, et al., 2011), regarding their protective roles as Ni chelators and detoxicants (Hasanuzzaman et al., 2019; Wen & Moriguchi, 2015). For example, the treatment of *Amaranthus* leaves with Put or Spd allowed these plants to grow well under Ni toxicity (Shevyakova, Cheremisina, & Kuznetsov, 2011) and in *Brassica napus* plants treated with Put, the ability of Ni accumulation in aerial organs has significantly increased with reduced toxic effects (Shevyakova et al., 2008), indicating that PAs accelerate the translocation of Ni, assuring its appropriate compartmentalization, and possibly improve the action of the AOX system against oxidative stress. In a study with *Amaranthus paniculatus* plants, Pietrini et al. (2015) found that the enhanced levels of free Spd and Spm contributed for alleviating the foliar effects of Ni on inducing oxidative stress and damage to the photosynthetic apparatus, when plants were exposed to moderate Ni

concentrations. Drastic and continuous accumulations of Pro or PAs have been noticed in *H. dubia* plants by Zhao et al. (2008) in response to Ni stress, confirming the effect that PAs have on Pro biosynthesis (Hasanuzzaman et al., 2019; Nasibi et al., 2013). Moreover, the demarked decrease in activity of PAO in response to (Zhao & Yang, 2008) is thought to be the dominating reason for the accumulation of Put in the leaves of sensitive plants (Zhao & Yang, 2008).

3.5 | Other metals—Manganese (Mn), iron (Fe), mercury (Hg), aluminium (Al), lead (Pb) and zinc (Zn)

As other metals, Mn is considered as an essential micronutrient for plant growth and development, being actively involved in several physiological mechanisms, being a co-factor of different enzymes, including AOX ones, and a structural component of the oxygen evolving complex in photosystem II (PSII), where it catalyses the water photolysis to generate the electrons needed to pump the photosynthetic reactions (Hussain, Nazir, & Fariduddin, 2019a; Schmidt et al., 2016). However, excessive amounts of this element quickly induce toxic effects at both macro- and cellular levels, disrupting protein folding and enzyme activity, inhibiting photosynthetic rates and disbalancing the normal mineral uptake (Fernando & Lynch, 2015; Hussain et al., 2019a). Despite the recognised toxicity of Mn to plants, not much is known regarding the influence of PA metabolism on its effects. Yet, according to Lei, Korpelainen, and Li (2007), the differential response recorded between two populations of the woody species *Populus cathayana* showed that the most tolerant one presented higher values of free PAs (Put, Spd and Spm), being this effect concomitant with an increased AOX activity and a lower accumulation of Mn. Similarly, as referenced in Table 1, the exogenous application of both 1 mM Put or Spd was found to alleviate Mn-induced stress in *B. juncea* plants, with a more prominent effect detected when Spd was sprayed (Hussain et al., 2019b). Based on their results, Mn alone decreased the biomass production and favoured the occurrence of oxidative damage; however, the foliar treatment with Spd or Put for 3 days helped to reduce metal accumulation and reduced the extent of redox disbalances (Hussain et al., 2019b). Moreover, the same research group also reported that the simultaneous application of Spd and 24-EBL resulted in synergic cooperation, contributing to an even improved tolerance of mustard plants to Mn-induced stress (Hussain et al., 2019a).

Paired with Mn, Fe is also an important micronutrient whose effects on plant growth are determined by its levels of occurrence. When present at adequate concentrations, Fe plays a major role in a diverse range of cellular and metabolic pathways, usually appearing in association with sulfur (S) complexes in proteins involved in photosynthesis, cellular respiration and mineral nutrients' assimilation (Aftab & Hakeem, 2020; Rawat, Nayan, Negi, Zaidi, & Arora, 2017). Nevertheless, problems associated with Fe bioavailability, either by its deficiency or excess, are commonly observed in field-grown plants (Aftab & Hakeem, 2020). Plants exposed to inadequate levels of this element have their growth retarded and undergo a pronounced state

of oxidative damage, because Fe deficiency and toxicity is usually associated with the occurrence of oxidative stress (Aftab & Hakeem, 2020). The involvement of PAs in Fe-induced physiological disorders are scarce, but those available point towards a positive relationship between PAs and plant tolerance to Fe. For instance, when studying the effects of Fe excess on the physiological status of two varieties of *Oryza glaberrima* Steud., Majerus, Bertin, and Lutts (2007) observed that the increased levels of free PAs (Put, Spd and Spm) highly contributed for an enhanced tolerance of the resistant genotype. In accordance, Ma, Duan, Yang, Yao, and Gao (2017) also found that the exposure of wheat seedlings to 300 μM Fe resulted in altered activities of PAO, but not DAO. Similarly, Zhu, Wang, Song, Zheng, and Shen (2016) reported that *Arabidopsis* mutants defective in Put biosynthesis (*adc2-1*) were hypersensitive to Fe deficiency when compared to the wild type (Col-1). However, when Put was exogenously supplied, mutant plants grew better and showed a higher tolerance. Moreover, a strong relationship between Put and NO was also described for Fe deficiency situations: Put-associated remobilization of Fe from roots is dependent on NO, because mutants lacking NO synthesis were not able to do so, even when Put was added (Zhu et al., 2016). Equivalent findings were also reported in *Solanum lycopersicum* L. plants grown under Fe deficiency (Zhou et al., 2019). Altogether, these data suggest that PA metabolism is, indeed, linked to Fe detoxification mechanisms.

Because of its non-essentiality to plants, Hg is very toxic at low levels and can cause oxidative stress and cellular damage, impairing growth and development. Alike other toxic HMs, Hg is targeted by defensive AOX molecules that plants produce to overcome this stress. Put has been reported to be produced and accumulated in water hyacinth leaves under Hg-stress, while the (Spd + Spm)/Put ratio decreased significantly (Ding et al., 2010). However, upon Spd (0.1 mM) treatment, the total levels of Put decreased, while the content of Spd and Smp increased, which contributed to a higher (Spd + Spm)/Put ratio, thereby resulting in a higher stress tolerance. Based on the results obtained, this shift observed between Put and (Spd + Spm) levels in response to the applied Spd relies on its interference with the activity of PAs-related enzymes, including ADC, ODC and PAO. Al induced toxicity is especially relevant in acidic soils, in which this metal is easily absorbed by plant roots causing inhibition in root cell growth and division, which hamper root growth and reduce the uptake of water and nutrients (Nahar et al., 2017). In a study with Al-stressed mung bean plants, Nahar et al. (2017) observed that exogenous application of Spd enhanced the endogenous levels of PAs, regulating the levels of Pro, preventing photosynthetic losses, and improving plant growth under Al stress. Once again, and confirming what is described for other HMs, Spd application boosted the AOX defences, lowered the degree of lipid peroxidation and the overproduction of ROS, namely O_2^- and H_2O_2 , and decreased the generation rate of methylglyoxal. Wen, Ban, Inoue, Matsuda, and Moriguchi (2009) reported that the overexpression of SPDS in European pear plants under Al-stress improved the survival status of the transgenic plants when compared to the wild type, because of modified activities of SOD and GR, and differential accumulation of Pro and MDA, in response to Al. In wheat, the activity of the cell wall bound PAO has increased under Al toxicity, leading to Spd oxidation

and H_2O_2 production (Yu et al., 2018). In contrast, Put application caused the inhibition of PAO activity, and subsequent reduction of H_2O_2 accumulation in roots under Al stress, suggesting an important protective role of Put against Al-induced oxidative stress (Yu et al., 2018).

Alongside, Pb-stress is usually associated to impaired plant growth and development, reduced chlorophyll production, thylakoid disorganisation and oxidative stress. Exposure to toxic levels of Pb was shown to decrease the level of thylakoid-bound Put and Put/Spm ratio in chloroplasts of barley leaves, along with a significant decrease in the number of thylakoids (Legocka, Sobieszczuk-Nowicka, Wojtyla, & Samardakiewicz, 2015). In this study, Legocka et al. (2015) suggested that thylakoid-associated PAs can regulate the response of the photosynthetic apparatus during Pb stress. Taie et al. (2019) found a positive correlation between PA application (0.25 mM Spm, 0.50 mM Spd or 1 mM Put), either by seed soaking or foliar spray, and the tolerance of wheat plants to Pb. According to their results, the most suitable approach was the seed priming with 1 mM Put, in which the best results were achieved. In general, the treatment with PAs stimulated plant growth under metal toxicity, contributing to a better redox status of the cells. Moreover, Pb-induced DNA fragmentation was counteracted by the applied treatments, accompanied by a decrease of Pb uptake. A previous work also conducted with *T. aestivum* also reported that all of the three main PAs (Spd, Spm and Put) can be a promising tool to alleviate Pb toxicity in crops, favouring the maintenance of the cellular redox homeostasis and the inhibition of metal uptake and accumulation (Rady et al., 2016).

Although it has an essential role as a micronutrient to plants, Zn can become a toxic HM above certain concentrations, inducing homeostatic imbalances, damage to cells and tissues and inhibition of growth. In a metabolomic approach, Rouphael et al. (2016) have found that the accumulation of Spd in lettuce plants under Zn stress was a specific response to this HM, presumably as the result of the activation of stress defensive mechanisms. On the other hand, (Shen, Zhang, & Zhang, 1998) had previously observed that while the Put levels increased in mung bean seedlings exposed to high levels of Zn, the levels of Spd and Spm were significantly reduced, and that the exogenous application of Put was not able to sustain growth in Zn stressed plants. In what concerns the exogenous application of PAs in Zn-stress plants, only one work has been conducted so far (Mir, Khan, & Fariduddin, 2015). The foliar application of 1 mM Spd in 15-day-old *Vigna radiata* plants exposed to Zn (200 mg/kg) helped to overcome the inhibitory effect of Zn on the growth traits, leaf water potential and photosynthesis. Moreover, the authors have also found that the interactive effects between 24-epibrassinolide and Spd were found to improve the tolerance of this plant species to the combined stress of salinity and Zn.

These examples allow us to conclude that PAs, particularly Spd and Spm, play an important role as modulators of plant's response mechanisms to HM-stress. Although it is not possible to conclude for each specific case which of the distinctive properties of which type of PAs was behind the response observed to the HM in question, it is likely that a Put burst serves as a signal, accusing the presence of stress (see Figure 3), and the differential accumulation of other PAs, along with the parallel production of other defence-related metabolites, makes it

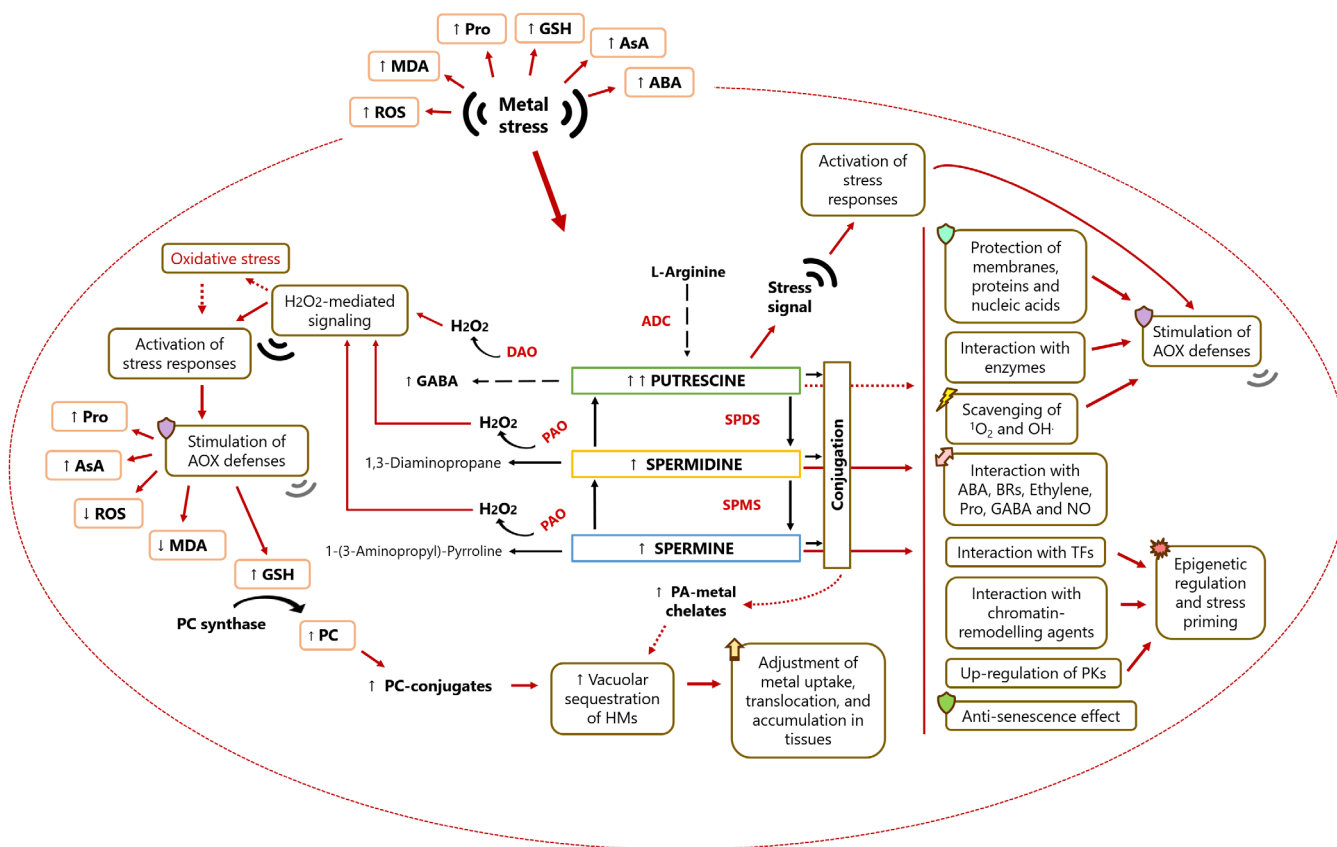


FIGURE 3 A mechanistic model on how PAs mediate plant responses to metal stress: Altered levels of phytohormones, induction of oxidative stress and activation of AOX defences are common responses in plants exposed to metal stress. As represented in this model, the metabolism of PAs can also be affected by HM, both at the level of synthesis and catabolism. Metal stress promotes the activity of the ADC, SPDS and SPMS enzymes, increasing the levels of Put in cells, which acts as a signal, accusing the presence of a stress factor, and, consequently, enhancing the biosynthesis of the other PAs, Spd and Spm. PAs can either act freely or conjugated to other biomolecules. Spd and Spm are the ones responsible for most regulatory functions of PAs, interacting with AOXs, phytohormones, transcription factors (TFs) and other molecules, affecting both stress responses, and basic cellular processes. PA catabolism is also enhanced in plants under metal-stress because of the increased activity of the DAO and PAO enzymes, resulting in a higher production of GABA and H_2O_2 . Eventually, high levels of PAs lead to the activation of AOX defences, either through H_2O_2 -mediated signalling, by direct scavenging of toxic ROS, interaction with AOX enzymes and/or protection of molecules from oxidation. The hypothesis that PAs form direct chelates with HM has also been incorporated in the model, although further studies are required to confirm this role. Still, the PA-induced enhancement of GSH content leads to a higher biosynthesis of PC-conjugates, which accelerates HM-detoxification

plausible to suggest that PAs are key players involved in various cellular processes and metabolic networks, both indirectly as signalling agents, and directly as protective elements in plant cells under stress. Knowing the exact mechanisms behind each PA-mediated regulation of plant stress responses and their interaction with other signalling molecules upon HM exposure is a complex matter (Figure 3) and further studies are required to establish optimised routes of PA application that could enhance plant performance under HM-stress.

4 | THE INVOLVEMENT OF PAS ON HM BIOACCUMULATION AND PHYTOREMEDIATION

The formation of Ni-PA chelates and their accumulation has first been reported in mammals' livers and kidneys and the formation of Put-Cu

(II), -Co(II), -Zn(II) and -Cd(II) complexes has also been established in vitro (Koutensky, Koutenska, Kotyzova, & Eybl, 1995). Moreover, as previously mentioned, a positive correlation between PAs and Ni detoxification was found in various studies by Shevyakova et al. (2008); Shevyakova, Cheremisina, and Kuznetsov (2011); Shevyakova, Il'ina, et al. (2011), who reported that when amaranth leaves were treated with exogenous PAs, the transport of Ni, as well as Fe, to leaves was enhanced (Shevyakova, Cheremisina, & Kuznetsov, 2011). Similarly, the same authors noticed an increased accumulation of Ni in the leaves of Put-treated rape plants (Shevyakova, Il'ina, et al., 2011). Such reports seem to suggest that PAs act as metal chelators in plants. In such view, the latter authors hypothesised that such chelates could be formed in the xylem tissues, which would allow for a greater translocation of Ni (and possibly other HMs) to the aboveground organs of plants, improving their efficiency for phytoextraction. This theory has not yet been corroborated, but is also represented in Figure 3.

In the above-mentioned study by Nahar et al. (2016), exogenously applied Spd reduced Cd content, accumulation, and translocation, as well as Cd-induced oxidative damage in *Vigna radiata* L. plants. However, all these authors have pointed out the powerful roles of PAs as members of the AOX system, protecting cells against oxidative stress, and most likely as enhancers of the PC content, which could justify their involvement in the detoxification of HMs. In fact, some researchers have reported that the positive effects exerted by PAs on plants under HM stress might not be related to direct metal chelation. For instance, the exposure of Indian mustard plants to high levels of Ni (up to 8 mM) led to an increased activity of DAO, supporting the connection between PAs catabolism and Ni-stress, but the authors suggested that PAs did not play a role as chelating agents for Ni (Sainger, Sharma, Baudh, Sainger, & Singh, 2014). Supporting this comes the fact that exogenous Spd applied to detached leaves of wheat exposed to Cd and Cu led to enhanced protection of membrane integrity, through lipid stabilisation and prevented the leakage of solutes under conditions of Cd- or Cu-induced oxidative damage (Groppa et al., 2007). These last reports do not confirm the involvement of PAs as HM chelators, but rather as AOXs and cellular protectors. Moreover, as mentioned in Section 3, PA's effects on HM homeostasis might be a mere consequence of enhanced levels of PCs (Hasanuzzaman et al., 2019; Nahar et al., 2016; Rady, Ahmed, Seif El-Yazal, & Taie, 2019), which is a well-recognised chelator important for the efficient detoxification of HMs. Overall, although there is a considerable amount of work elucidating the effects of PAs on HM tolerance, translocation and accumulation, there is still not enough evidence to address PAs as metal chelators.

5 | CONCLUSIONS AND PERSPECTIVES

It is well settled that the PAs' main way of action relies on their protective roles as stabilisers and AOXs, which are transversal features to most plant species and stress conditions. In this way, exogenous application of PAs may work as an efficient tool to increase plant tolerance against HM-induced injuries in plants, which can directly affect plant survival and its ability to uptake and accumulate higher amounts of HM in its tissues, because of a longer life span. However, from a phytoremediation perspective, the few studies reporting to their participation as HM chelators suggest that such role is rather species-, tissue- and/or HM-specific and is probably determined by the plant's life cycle and growth stage. Further research is still needed to clarify the alleged role of PAs as HM chelators within plant body and cells. Such information could be crucial for the improvement of phytoremediation strategies or for the safe cultivation of plants in HM-polluted soils.

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CONFLICT OF INTEREST

The authors also declare that there is no conflict of interest.

ORCID

Cristiano Soares  <https://orcid.org/0000-0003-3330-2024>

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